TERNARY COMPLEXES IN SOLUTION.* BRIDGING OF THE STACKED ADDUCT BETWEEN TRYPTOPHAN AND ADENOSINE 5'-TRIPHOSPHATE BY ZINC(II)

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1. Introduction

It is known that DNA and RNA may interact with proteins. One of the driving forces leading to adduct formation between these macromolecules is the stacking between the bases of the nucleic acids and the aromatic moieties of the amino acid residues of the proteins [2–4]. In accord herewith, it could be shown that the corresponding monomeric units interact also with each other, e.g. nucleotides or nucleosides form pseudo-charge–transfer complexes with tryptophan [5,6] and other aromatic amino acid derivatives [7,8].

Recently, it has been demonstrated that such stacked or chargetransfer complexes could be stabilized by forming a metal ion-bridge between the two constituents of such an adduct [9,10]. The ligands used in this study were 2,2'-bipyridyl and adenosine or inosine 5'-triphosphate; in the resulting ternary Cu^{2+} complexes dominates the structure which allows stacking between the aromatic amine and the purine moiety. As the potential occurrance of such stacked metal ion-bridged complexes in biological systems is very high, for example in the mentioned nucleic acid-protein systems, or in synaptosomes [11,12] which contain aromatic (biogenic) amines, ATP and several metal ions like Fe^{3+}, Cu^{2+}, Mg^{2+} and Zn^{2+}, we felt it is desireable to do a model study by using constituents that all occur in nature.

In fact, by means of NMR spectroscopy it is possible to show that in the mixed-ligand complex, ATP–Zn^{2+}–tryptophan, a stacking occurs between the indole and purine residues of the metal ioncoordinated ligands. Since the effect of Zn^{2+} on the PMR spectra of ATP and tryptophan is predominantly on shift and only very slightly on line width [13], this metal ion appeared most suitable for this study.

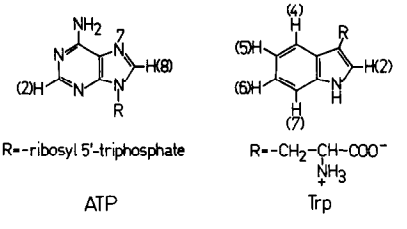
2. Materials and methods

The disodium salt of adenosine 5'-triphosphate was obtained from Serva Feinbiochemica GmbH, Heidelberg (Germany). Tryptophan, D_2O, and zinc perchlorate were from Fluka AG, Buchs (Switzerland).

Measurements were made with a Bruker WH-90 FT spectrometer with sodium 3-(trimethylsilyl)propane sulfonate as an internal standard. The p*D* of the D_2O solutions containing the reagents was adjusted to 9.40 ± 0.05 (p*D* = pH-meter reading +0.4) [14] by dotting with a glass stick and concentrated NaOD.

3. Results and discussion

The NMR signals of ATP and tryptophan were identified according to Cohn and Hughes [15] and Bak et al. [16], respectively. The numbers given below at the protons in the formulas of ATP and tryptophan



* Part 21 of the series. Part 20 appeared elsewhere [1].

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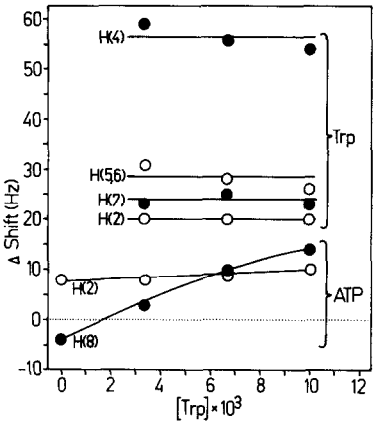

Fig. 1. Change in chemical shift, Δ shift, of the PMR-resonances for the protons H (2) and H (8) of ATP and for H (2), H (4), H (5,6) and H (7) of tryptophan in D_2O at p*D* 9.4 in the presence of Zn^{2+} in dependence on the total concentration of tryptophan. [ATP] = $[Zn(C10_4)_2]$ = 10^{-2} M; room temperature (~ 25° C). The chemical shifts of the free ligands are for H (2) and H (8) of ATP 742 and 769 Hz, respectively, and for H (2), H (4), H (5,6) and H (7) for tryptophan 665, 680, 652 and 699 Hz, respectively. In case of multiplets the data refer to the mid point of the pattern.

correspond to the labels inserted in fig. 1. The difference in the chemical shift, i.e. Δ shift, was obtained by subtracting the chemical shift observed in the presence of complexing agents from the chemical shift measured for the free ligands. This means a positive value of Δ shift implies an upfield shift.

Fig. 1 shows the relationship between increasing amounts of tryptophan and the change in chemical shift, Δ shift, of the H (2) and H (8) protons of ATP and the H (2), H (4), H (5,6) and H (7) protons of tryptophan. The large Δ shifts, especially of H (4), observed for the protons of tryptophan can unfortunately not be discussed in an unequivocal manner as the influence of Zn^{2+} on tryptophan (i.e., the binary system) could not be determined due to precipitation. The influence of ATP in the absence of Zn^{2+} on the chemical shifts of the tryptophan-protons is small; we measured in 10^{-2} M solution for H (2), H (4), H (5,6) and H (7) an upfield shift of 10, 6, 4, and 6 Hz, respectively. This indicates a stacked adduct [7,8] of weak stability and is in accord with the small upfield shifts observed for H (2) (2 Hz) and H (8) (2 Hz) of ATP in the same system.

The constant size of Δ shift for the tryptophan protons (cf. fig. 1) is in accord with the expectation that at p*D* 9.4 the mixed-ligand Zn^{2+} complex is formed to a large extent. Additionally, it should be noted that the signals due to H (4) and H (5,6) are somewhat overlapping what leads to an increased error in the evaluation of Δ shift for these protons.

However, the Δ shift of the H (2) and H (8) protons of ATP demonstrates unequivocally the formation of a rather stable Zn^{2+}-bridged stacked adduct between the indole and purine moieties of ATP and tryptophan, respectively. Especially the change of the chemical shift of H (8) under the influenc of tryptophan in the presence of Zn^{2+} is very significant. It is worth to recall here that increasing amounts of Zn^{2+} shift the signal of H (8) just in the opposite direction [13].

Furthermore, the present experimental data suggest that the intramolecular stacking within the ATP–Zn^{2+}–tryptophan complex occurs predominantly between the imidazole part of the adenine moiety and the benzene ring of the indole residue. Finally, the NMR data assembled for the ATP–Co^{2+}-*l*-adrenaline system [17] may be analogously understood, i.e. by the formation of a Co^{2+}-bridged stacked adduct between the purine moiety of ATP and the catechol ring of *l*-adrenaline.

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